

PHYTOCHEMICAL ANALYSIS OF *VERNONIA ADOENSIS* LEAVES AND ROOTS USED AS A TRADITIONAL MEDICINAL PLANT IN KENYA

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ABSTRACT

Medicinal plants play an important role in the treatment of various sicknesses in Kenya and the whole world. Vernonia adoensis is one of such plants used traditionally in Kenya to treat illness such as gonorrhea. The main aim of conducting this study was to analyze the photochemical composition of the plant. From this study, phenols, saponnins, flavonoids, glycosides, tannins were found on both roots and leaves of the plant, while alkaloids and terpenoids were found on the leaves but absent in the roots. Anthroquinones were found to be absent on leaves and roots of the plant.

KEY WORDS

Vernonia Adoensis, Phytochemical, Medicinal herbs

INTRODUCTION

The use of medicinal plants to treat diseases is as old as man. Medicinal plants have been used since ancient times to treat many illnesses. The importance of these plants lies on certain chemical components of the plants which are known as phytochemicals. Mir (2013). Research has shown that the concentration of these compounds in plants is directly related to their capability to treat certain illness. Many of these non-nutritive secondary metabolites are found in plants which are even used for food. According to Cousins (2002) over 80% of the plants in Nigeria are used for treatment of malaria and other sicknesses are also used as food, there seem to be no much distinction between medicinal benefits of plants and there nutritive value.

The published WHO traditional strategy addressed the issues and provided a framework for countries to develop policies to govern medicinal plants use.

The strategy put forward by WHO advocates the formulation of a policy by states as the first component of developing traditional medicine, India is one of the few countries which has started to

develop such policies (Prajapati, 2003). Over past few years much research has being done and is still going on to prove scientifically the plants nutritional value and medicinal value. A good number of chemical compounds have being discovered from plants and found to have pharmacological value, this has lead to the development of over 25% of all the artificial medicines used today. Many of the traditional medicinal plants species used all over the world have being found to have great pharmacological value. Studies carried out throughout Africa confirm that indigenous plants are the main constituents of traditional African medicines.

Over 80% of the people in developing countries use medicinal plants to treat the illnesses which affect them in daily basis (Ganga, 2012). This can be attributed to poverty in these countries which has lead to inefficient health care system in hospitals and inadequate resources to access these facilities. People in these countries look for cheap and available medicines which are known traditionally to cure the illnesses. The use of herbal medicines in the western world is steadily growing with 40% of the population

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using plants to treat illnesses, while in Kenya 90% of the population have one time in their life used medicinal plants (Adongo et al, 2012). The use of these plants in treatment of ailments is mainly based on the type of flora in that region.

Our environment is very rich with a great range of medicinal plant and this mainly explains the reason why our grand's lived for quite some time. They could stay in the bush during war for some time and even could use plants to treat ailments and wounds affecting soldiers in the battle ground. People all over the world should look around them especially in Africa were this information has not completely being replaced by industrial medicines, lest we forget this important aspect of treatment. In many communities in Africa they still consider the use of medicinal plants as an important part of their culture, just to mention, the maasai community in Kenya still value their culture very much, the kalenjn community and their medicinal fermented milk which is prepared mainly from medicinal plants such as Senna didymobotrya stem which previous studies have shown this plant to have a great potential in treatment of diseases such typhoid, diarrhea and food poisoning caused by Bacillus (Ngule, 2013). According to Kokwaro (2009) the reason why herbal medicine still remains a matter of argument is because of some greedy practitioners who want to become wealthy by pretending to know much about the treatment of every disease that their clients complain about. This has lead to administration of wrong drugs which do not cure a patient leading to death of the individual. Proper scientific evidence needs to be provided in order to create confidence in medicinal herbs. The increase of multi-resistant strains of bacteria calls for new discoveries antibacterial classes and chemical compounds that can clearly inhibit these resistant strains, this is the reason why much research should turned to plants which have being since ancients times used to treat many diseases.(Cousins et al, The non-nutritive plant components are referred to as phytochemicals, which can be divided in two major categories which are primary and secondary, with the primary constituting of carbohydrates, proteins and chlorophyll and the secondary consisting of tannins, alkaloids, saponins, steroids, flavonoids, terpenoids and anthroquinones (Maobe, et al, 2013). The secondary metabolites help the plant survive in the environment by protecting them against predators but research has shown that these metabolites can be used to treat diseases in both animals and humans(Kokwaro,2009). Physiological activities of phytochemicals have being found to include cancer prevention, antibacterial, antifungal, antioxidative, hormone action and enzyme

A big percentage of plants in the savanna and semiarid areas of east Africa where Kenya is located contains alkaloids which have been associated with increase in renal secretion when ingested hence used as a diuretics and in the treatment of dropsy (Kokwaro, 2009). According to Mir (2013) the use of alkaloids, saponins and tannins as antibiotics has been scientifically justified.

stimulation.

Majority of the pharmacologically active chemical compounds were found mainly in ethanol extracts which is contrary to previous researches which had affirmed the traditional way of extracting these compounds using water (Iqbal, 2012).

Vernonia adoensis is used traditionally by many communities to treat various illnesses due to lack of resources to access hospitals or even preference of the use of medicinal plants. The plant roots are used mainly for the treatment of sexually transmitted disease called gonorrhea by people in the Rift valley and Western part of Kenya (Kokwaro, 2009). The plant leaves are used in the treatment of malaria. The decoction of the roots mixed with the bark of other trees is used in the treatment of heart and kidney problems (Kokwaro, 2009). According to the study carried out by Stangeland et al (2010) the plant had a very high anti-plasmodia activity and the leaves are used to treat T.B. Much research has not being done to test the phytochemical analysis of this plant. This study was carried out to investigate the presence of phytochemicals in the plant.

MATERIALS AND METHODS

Sample Collection and Preparation: The herb Vernonia adoensis was randomly collected in the natural forest around Baraton University in Nandi County. The samples were collected and identified by a taxonomist in the Biology Department, Baraton

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University. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

Extraction procedure: Using electric analytical beam balance fifty grams of the powdered leaves of the Vernonia adoensis was placed in 1000ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the leaves were completely submerged in the solvent. The mixture was then agitated for thorough mixing. The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no 1 filter paper and a vacuum and pressure pump. The filtrate was refiltered again using the same apparatus. The solvent was evaporated using rotary vacuum evaporator (R-11) with a water birth at 40°C. The extract was brought to dryness using vacuum and pressure pump at room temperature. The residue was then obtained and used for the experiment.

Qualitative phytochemical analysis: The extracts phytochemical analysis for identification of bioactive chemical constituents was done using standard procedures by Trease (1989) and Evans (1989), Harborne (1973) and Sofowara (1983).

1. Tannins

About 0.5 g of the sample was put in a test tube and 20 ml of distilled water was added and heated to boiling. The mixture was then filtered and 0.1 % of FeCl₃ was added to the filtrate and observations made. A brownish green color or a blue black coloration indicated the presence of tannins.

2. Saponins

The crude solvent extract was mixed with 5 ml of water and vigorously shaken. The formation of stable form indicated the presence of saponins.

3. Flavonoids

About 1g of the plant extract was mixed with a few fragments of magnesium ribbon (0.5 g) and a few drops of concentrated hydrochloric acid were added. A pink or magenta red color development after 3 minutes indicated presence of flavonoids.

4. Tarpenoids

The solvent extracts of the plant material was taken in a clean test tube 2 ml of chloroform was added and

vigorously shaken, then evaporated to dryness. To this, 2 ml of concentrated sulphuric acid was added and heated for about 2 minutes. A grayish color indicated the presence of tarpenoids.

5. Glycosides.

- a. Salkowsks' test: The solvent extract of the plant material was mixed with 2 ml of chloroform and 2ml of concentrated sulphuric acid was carefully added and shaken gently, then the observations were made. A red brown colour indicated the presence of steroidal ring (glycone portion of glycoside)
- b. Liebermanns test: The solvent extract of the plant material was mixed with 2 ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice and observations made. A color change from violet to blue to green indicated the presence steroidal nucleus (glycone portion of the glycosides)
- c. **Keller-kilani test:** The solvent plant material extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl₃ the mixture was then poured into a test tube containing 2ml of concentrated sulphuric acid. A brown ring at the interface of the two solutions indicated the presence of cardiac glycoside.

6. Alkaloids

The crude extract was mixed with 1% of HCl in a test tube. The test tube was then heated gently and filtered. To the filtrate a few drops of Mayers and Wagner's reagents were added by the side of the test tube. A resulting precipitate confirmed the presence of alkaloids.

7. Steroids

Libermann Burchard reaction: About 2g of the solvent extract was put in a test tube and 10 ml of chloroform added and filtered. 2 ml of the filtrate was mixed with 2 ml of a mixture of acetic acid and concentrated sulphuric acid. Blue green ring indicated the presence of steroids.

8. Phenols

The plants solvent extract was put in a test tube and treated with a few drops of 2% of FeCl₃. Blue green or black coloration indicated the presence of phenols.

9. Anthroquinones

Borntregets test: About 5 gm sample of the extract was put in a test tube and 10 ml of benzene added.



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The mixture was shaken and filtered. 5 ml of ammonia solution was added to the filtrate and the mixture shaken. Presence of violet color in the

ammonical phase (lower phase) indicated the presence of anthroquinones.

RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of Vernonia leaves

Phytochemical	Observation	Inferences
Terpenoids	Grayish color	Terpenoids present
Steroidal ring	Red-brown color	Steroidal ring present
Steroidal nucleus	No change of violet, blue to	Steroidal nucleus absent
	green color	
Flavonoids	Magenta-red color	Flavonoids present
Alkaloids	Precipitate observed	Alkaloids present
Steroids	Blue- green ring observed	Steroids present
Phenols	Blue-green color observed	Phenols present
Anthroquinones	Absence of violet color at the	Anthroquinones absent
	lower phase	
Tannins	Blue-black color observed	Tannins present
Saponins	Stable foam	Saponins Present

Table 2: Phytochemical analysis of Vernonia roots

Saponins	Stable foam observed	Saponins present
Flavonoids	Magenta-red color	Flavonoids present
Tannins	Presence of blue black color	Tannins present
Terpenoids	Absence of grayish color	Terpenoids absent
Glycosides (Salkowsk's test).	Presence of a red-brown color	Glycosides present
Glycosides(Liebermann's test)	Presence of blue-green ring	Steroidal nucleus present
Glycosides(Keller –Kilani test)	A brown ring at the interphase	Cardiac glycoside present
Alkaloids	Precipitate absent	Alkaloids absent
PhenoIs	Presence of blue-green color	Phenols present
Steroids	Absence of a blue-green color	Steroids absent
Anthroquinones	Absence of violet color at the interphase.	Anthroquinones absent

The presence of Saponins shows the potential of the plant to be used to produce mild detergents and in intracellular histochemistry staining to allow antibody access to intercellular proteins (Maobe, 2013). They have being found to treat hypercholesterolemia,

hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anticancer and weight loss (Maobe, 2013). They are used to stop bleeding, treating wounds and ulcers as it helps red blood cells to precipitate and coagulate (Okwu, et al,

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2006). They can reduce the uptake of some nutrients such as glucose and cholesterol at the gut causing hypocholesterolemic effect and thereby relieve the liver metabolic burden (Smirth, 2009). This can be attributed to ability of saponins to bind with glucose and cholesterol molecules. Saponins have also being associated with inhibitory effect on inflammatory (Just et al, 1998).

Alkaloids which are secondary metabolites, they can be defined as a cyclic compound which has nitrogen in a negative oxidation state. They affect the chemical transmitters' action of the nervous system. They also have other pharmacological activities such as analgesic, antispasmodic, antihypertensive effects and antiarrhymic effects and antibacterial. According to Karoo (2005) alkaloids have good antibacterial activity with highest zone of inhibition recorded on gram-positive bacteria. Cryptolepine a major alkaloid in S.acuta was found to be an antimalarial agent (Banzouzi et al, 2004). Cryptolepine has also being used clinically to treat malaria, colic and stomach ulcers (Boye et al, 1983) and also used in anticancer drugs (Bonjean et al, 1998). According to Karou (2006) much study has being done on pharmacological properties of alkaloids on antiprotozoal, cytotoxic and anti-inflammatory properties.

Tannins are also secondary metabolites in plants. They are glycosides of gallic or protocatechvic acids. There astringent property makes them useful in preventing diarrhea and controlling hemorrhage due to their ability to precipitate proteins, mucus and constrict blood vessels (Kokwaro, 2009). This is the reason why traditional healers use plants reach in tannins to treat wounds and burns since they are able to cause blood clotting. Some tannins have being reported to inhibit HIV replication selectively besides the use of diuretics(Argal, 2006). This shows how traditional medicinal plants rich in tannins can be used to control this dangerous disease. Tannins have also shown antiparasitic effects (Akiyama, 2004). According to Bejal et al (1988) tannins can also be used to protect the kidney since when taken the poliovirus, herpes complex virus and various enteric viruses are inactivated. It has also being discovered that tannins have anti-inflammatory and antiulcer activity in rodents, (Aquino, 2006), a clear indication that they can be used to treat against inflammation.

Foods rich in tannins can be used to treat hereditary hemochromatosis which is a hereditary disease characterized by excessive absorption of dietary iron. According to Chung (1998) many tannin molecules have been shown to reduce the mutagenic activity of a number of mutagens. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property which is important in protecting cellular oxidative damage including lipid peroxidation. The growth of many fungi, yeast, bacteria and viruses has being proven to be inhibited by tannins. Tannins have also been reported to exert physiological effects such as to accelerate blood pressure, decrease the serum lipid level, produce liver necrosis and module immunoresponses. The dosage and kind of tannins are critical to these effects (Chung, 1998).

Flavonoids secondary metabolites with are polyphenolic structure and synthesized in plants through polypropanoid pathway (Ghasemzadeh, 2011). Flavonoids have being classified in to six subgroups which include flavones, flavanol, flavanone, flava-3-ols, isoflavone and anthocynidin. Flavonoids are known to contain specific compounds called antioxidants which protect human, animal and plant cells against the damaging effects of free radicals. Imbalance between free radicals and antioxidants leads to oxidative stress which has being associated with inflammation, autoimmune diseases, cataract, Parkinson's cancer, disease, aging arteriosclerosis. It also plays a role in heart diseases and neurodegenerative diseases. Flavonoids have also vasodilator activity a property which is useful in improving blood circulation in brain and in Alzheimer disease (Sharma, 2006). Leaf extract of Ginkgo biloba which contains flavonoids was used for improving blood circulation in brain varix. Several isoflavone can used to improve blood circulation. Furanocoumarins can alter hexobarbital induced sleeping time and showed cytotoxic action and hence inhibited growth of tumor in mice. Free radicals including the hydroxyl, hydrogen peroxide, superoxide and lipid peroxide have being associated with a number of diseases such as cardiovascular disease. cataracts. diabetes. gastrointestinal inflammatory diseases, cancer, asthma, liver disease, macular degeneration, periodontal disease and other

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inflammatory processes. These oxidants are produced during normal body chemical processes. They can be damaged through free-radical damage. Flavonoids such as quercetin, rotin, catechin and its derivatives and the oligomeric proathocyanidins (OPCS) have shown in vitro studies to inhibit the oxidation of low-density lipoproteins (LDL).

Glycosides another type of secondary metabolites are organic compounds from plants or animal sources in which a sugar is bound to a non-carbohydrate moiety. The term Glycoside is a collective term used for compounds formed with a glycosidic bonding between a sugar and another compound other than sugar. Cardiac glycosides have being used traditionally as arrow poisons or as heart drugs. They are used to strengthen the heart and make it function properly under controlled therapeutic dose. Cardiac glycosides bind to and inhibit Na⁺/K⁺-ATPase, inhibition of N+/K+-ATPase raises the level of sodium ions in cardiacmyocytes, which leads to an increase in the level of calcium ions and an increase in cardiac contraction force (Schatzmann, 1965). unexpected results relating cardiac glycosides with anticancer properties has created a great interest in this secondary metabolite. This has lead to clinical trial of cardiac glycosides based drugs in clinics (Newmann, 2008).

CONCLUSION

The presence important pharmacological phytochemicals in the plant leaves and roots is an indication of the diverse medicinal importance of *Vernonia adoensis*. phenols, saponins, flavonoids, glycosides, tannins were found on both roots and leaves of the plant, while alkaloids and terpenoids were found on the leaves but absent in the roots. Anthroquinones were found to be absent on leaves and roots of the plant. More research needs to be done to identify the specific compounds and there structures and also the antibacterial activity of the plant.

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